S/N 10/815,262

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant: | John F. Engelhardt et al. | Examiner: | Kevin Hill |
| Serial No.: | 10/815,262 | Group Art Unit: | 1633 |
| Filed: | March 31, 2004 | Docket No.: | 875.074US1 |
| Customer No.: | 21186 | Confirmation No.: | 7471 |

Title: COMPOUNDS AND METHODS TO ENHANCE rAAV TRANSDUCTION

## SUPPLEMENTAL RULE 132 DECLARATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

We, Dr. John Engelhardt and Dr. Ziying Yan, declare and say as follows:

1. We are two of the named co-inventors of the above-identified application and we make this Declaration in support of the patentability of the pending claims.

2. In the Office Action mailed August 6, 2009 for the above-referenced matter, the Examiner asserted that the practice of the claimed methods would require undue experimentation because, among other things, the specification does not teach *in vivo* dosing and dosage regimens to avoid toxicity.

3. As discussed in the Amendment which accompanies this Supplemental Declaration, it is well within the skill of the art worker to select nontoxic doses of one or more compounds and suitable routes of administration for those compounds. In particular, with regard to nontoxic doses and suitable routes of administration for certain compounds useful to enhance recombinant adeno-associated virus (rAAV) transduction, the Examiner is requested to consider the following.

4. A toxicity study conducted by one of skill in the art might include administering to a non-human mammal one or more doses of a selected compound by a particular route. Then, at one or more times after that administration, tissue samples from those non-human mammals and controls would be harvested and analyzed.

5. A pilot toxicity study was conducted to assess the potential cytotoxicity of administering Doxil® (200 μM), Z-LLL (40 μL) or Doxil® (200 μM) and Z-LLL (40 μL), compounds that were found to enhance rAAV transduction, to mouse lung. Phosphate buffered saline was used as a negative control. Samples were collected at days 1, 14 and 30 post-administration and initially read blind as to treatment.

6. When the samples were assessed without reference to treatment, almost all animals in all groups had very minimal (background) levels of perivascular or interstitial inflammation. The observed background level of inflammation would not be expected to have any clinical significance and is common in rodent colonies.

7. When the samples were interpreted in view of the negative control samples, a few animals at 30 days post-administration had minor inflammatory changes that were not clinically significant and not consistently observed within a treatment group. Those changes were thus not interpreted as being test material related (one in fact was from a negative control animal). Although three animals administered Z-LLL had multifocal areas of necrosis and neutrophilic inflammation often centered in distal airways or within alveolar spaces at day 14 post-administration, these changes were not present in that treatment group on day 1 or day 30, nor were they observed in animals receiving a combination of Doxil® and Z-LLL. It was concluded that if the observed lesions were reproducible, they may have clinical consequence in animals already experiencing respiratory issues.

8. Similar studies to those discussed in detail above can be done using other compounds, other doses or other routes of administration, or combinations thereof, to assess potential toxicity. Moreover, those types of studies are routine in the art and allow the art worker to select nontoxic amounts and routes of administration of a compound to achieve a particular outcome.

9. We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further

that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 12/4/09 By: _____
Dr. John Engelhardt

Dated: 12-4-09 By: _____
Dr. Ziying Yan

J Gene Med. 2005 Nov;7(11):1429-38.
Adeno-associated virus serotypes 1 to 5 mediated tumor cell directed gene transfer and improvement of transduction efficiency.
Hacker UT, Wingenfeld L, Kofler DM, Schuhmann NK, Lutz S, Herold T, King SB, Gerner FM, Perabo L, Rabinowitz J, McCarty DM, Samulski RJ, Hallek M, Büning H.
Klinik für Innere Medizin I, Klinikum der Universität zu Köln, Joseph-Stelzmann-Strasse 9, 50925 Köln, Germany.
BACKGROUND: Gene therapy is an attractive new approach for the treatment of cancer. Therefore, the development of efficient vector systems is of crucial importance in this field. Different adeno-associated virus (AAV) serotypes have been characterized so far, which show considerable differences in tissue tropism. Consequently, we aimed to characterize the most efficient serotype for this application. METHODS: To exclude all influences other than those provided by the capsid, all serotypes contained the same transgene cassette flanked by the AAV2 inverted terminal repeats. We systematically compared these vectors for efficiency in human cancer cell directed gene transfer. In order to identify limiting steps, the influence of second-strand synthesis and proteasomal degradation of AAV in a poorly transducible cell line were examined. RESULTS: AAV2 was the most efficient serotype in all solid tumor cells and primary melanoma cells with transduction rates up to 98 +/- 0.3%. Transduction above 70% could be reached with serotypes 1 (in cervical and prostate carcinoma) and 3 (in cervical, breast, prostate and colon carcinoma) using 1000 genomic particles per cell. In the colon carcinoma cell line HT-29 proteasomal degradation limited AAV1-AAV4-mediated gene transfer. Moreover, inefficient second-strand synthesis prevents AAV2-mediated transgene expression in this cell line. CONCLUSIONS: Recent advances in AAV-vector technology suggest that AAV-based vectors can be used for cancer gene therapy. Our comparative analysis revealed that, although AAV2 is the most promising candidate for such an application, serotypes 1 and 3 are valid alternatives. Furthermore, the use of self-complementary AAV vectors and proteasome inhibitors significantly improves cancer cell transduction.

Gene Ther. 2005 Oct;12(20):1534-8.
Adeno-associated virus (AAV)-7 and -8 poorly transduce vascular endothelial cells and are sensitive to proteasomal degradation.
Denby L, Nicklin SA, Baker AH.
British Heart Foundation, Glasgow Cardiovascular Research Centre, Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.
Transduction of the vascular endothelium by adeno-associated virus (AAV) vectors would have broad appeal for gene therapy. However, levels of transduction by AAV serotype-2 are low, an observation linked to deficiencies in endothelial cell binding, sequestration of virions in the extracellular matrix and/or virion degradation by the proteasome. Strategies to improve transduction of endothelial cells include AAV-2 capsid targeting using small peptides isolated by phage display or the use of alternate serotypes. Previously, we have shown that AAV serotypes-3 through -6 transduce endothelial cells with poor efficiency. Recently, AAV serotypes-7 and -8 have been shown to mediate efficient transduction of the skeletal muscle and liver, respectively, although their infectivity profile for vascular cells has not been addressed. Here, we show that AAV-7 and -8 also transduce endothelial cells with poor efficiency and the levels of transgene expression are markedly enhanced by inhibition of the proteasome. In both cases proteasome blockade enhances the nuclear translocation of virions. We further show that this is vascular cell-type selective since transduction of smooth muscle cells is not sensitive to proteasome inhibition. Analysis in intact blood vessels corroborated these findings and suggests that proteasome degradation is a common limiting factor for endothelial cell transduction by AAV vectors.

Biochem Biophys Res Commun. 2005 Jun 17;331(4):1392-400.
Proteasome modulating agents induce rAAV2-mediated transgene expression in human intestinal epithelial cells.
Tang SC, Sambanis A, Sibley E.
Division of Pediatric Gastroenterology, Stanford University School of Medicine, Stanford, CA 94305, USA.
Intestinal gene transfer offers promise as a therapeutic option for treatment of both intestinal and non-intestinal diseases. Recombinant adeno-associated virus serotype 2, rAAV2, based vectors have been utilized to transduce lung epithelial cells in culture and in human subjects. rAAV2 transduction of intestinal epithelial cells, however, is limited both in culture and in vivo. Proteasome-inhibiting agents have recently been shown to enhance rAAV2-mediated transgene expression in airway epithelial cells. We hypothesized that similar inhibition of proteasome-related cellular processes can function to induce rAAV2 transduction of intestinal epithelial cells. Our results demonstrate that combined treatment with proteasome-modulating agents MG101 (N-acetyl-L-leucyl-L-leucyl-L-norleucine) and Doxorubicin synergistically induces rAAV2-mediated luciferase transgene expression by >400-fold in undifferentiated Caco-2 cells. In differentiated Caco-2 monolayers, treatment with MG101 and Doxorubicin induces transduction preferentially from the basolateral cell surface. In addition to Caco-2 cells, treatment with MG101 and Doxorubicin also results in enhanced rAAV2 transduction of HT-29, T84, and HCT-116 human intestinal epithelial cell lines. We conclude that MG101 and Doxorubicin mediate generic effects on intestinal epithelial cells that result in enhanced rAAV2 transduction. Use of proteasome-modulating agents to enhance viral transduction may facilitate the development of more efficient intestinal gene transfer protocols.

Arch Dermatol Res. 2005 May;296(11):528-35. Epub 2005 Mar 18.
Recombinant adeno-associated virus type 2-mediated gene transfer into human keratinocytes is influenced by both the ubiquitin/proteasome pathway and epidermal growth factor receptor tyrosine kinase.
Braun-Falco M, Eisenried A, Büning H, Ring J.
Klinik und Poliklinik für Dermatologie und Allergologie am Biederstein, Division of Environmental Dermatology and Allergy, National Research Center for Environment & Health, Technische Universität München and GSF, Munich, Germany. mbf@lrz.tum.de
Efficient gene delivery into keratinocytes is a prerequisite for successful skin gene therapy. Vectors based on recombinant adeno-associated virus type 2 (rAAV-2) offer several promising features that make them attractive for cutaneous applications. However, highly efficient gene delivery may be hampered by different cellular factors, including lack of viral receptors, impairment of cytoplasmic trafficking or limitations in viral second-strand synthesis. This study was undertaken to find factors that influence rAAV-2-mediated in vitro gene transfer into human keratinocytes and, consequently, ways to optimize gene delivery. Transduction experiments using rAAV-2 vectors expressing green fluorescent protein (GFP) demonstrated that impaired cellular trafficking of vector particles and high levels of autophosphorylation at epidermal growth factor receptor tyrosine kinase (EGF-R TK) have a negative influence on gene transfer into keratinocytes. Treatment of keratinocytes with proteasome inhibitor MG132 resulted in a transient augmentation of GFP expression in up to 37% of cells. Treatment with EGF-R TK inhibitors (quinazoline type) enhanced transgene expression in 10-14.5% of the cells. Gene expression was stable for more than 10 weeks and persisted until proliferative senescence occurred. This stable gene expression allows speculation that keratinocyte stem cells have initially been transduced. These findings might have relevance for the use of rAAV-2 vectors in skin gene therapy: transient enhancement of rAAV-2 transduction with proteasome inhibitors might be useful for genetic promotion of wound healing or skin-directed vaccination. Treatment with quinazolines may increase rAAV-2 transduction of keratinocyte stem cells, which is important for gene therapy approaches to inherited diseases.

Mol Ther. 2005 Apr;11(4):600-7.
Proteasome inhibition enhances AAV-mediated transgene expression in human synoviocytes in vitro and in vivo.
Jennings K, Miyamae T, Traister R, Marinov A, Katakura S, Sowders D, Trapnell B, Wilson JM, Gao G, Hirsch R.
William S. Rowe Division of Rheumatology, Children's Hospital Medical Center, Cincinnati, OH 45229, USA.
To explore the potential applicability of recombinant adeno-associated virus (rAAV) vectors in the treatment of rheumatoid arthritis (RA), primary human fibroblast-like synoviocytes (FLS) derived from patients with RA were infected with rAAV encoding mouse IL-10 under the control of the CMV promoter. Addition of the proteasome inhibitor carbobenzoxy-l-leucyl-l-leucyl-l-leucinal (zLLL) to the cultures dramatically enhanced expression of the IL-10 transgene, in a dose-dependent manner. The increased expression was transient, peaking at 3 days and returning to near baseline by 7 days. The enhancement was observed even when zLLL was added 13 days after infection with rAAV. The effect of zLLL was not specific to either the mIL-10 transgene or the CMV promoter, as similar findings were observed using an rAAV construct encoding alpha1-anti-trypsin under the control of the chick beta-actin promoter or GFP, driven by the CMV promoter. Transgene expression could be repeatedly induced by reexposure to zLLL. Transgene mRNA levels increased in parallel with protein levels. Transgene expression could also be repeatedly induced in vivo by administering zLLL to SCID mice previously injected with rAAV-infected FLS. These data demonstrate that proteasome inhibition can dramatically enhance transgene expression in human RA FLS following infection with rAAV and suggest a possible approach to regulating synovial transgene expression in vivo.

Abstract1 (full article not available)

Thrombosis and Haemostasis 2009; Volume 7, Supplement 2: Abstract OC-TH-087

Adeno-associated virus vector expression of large genes is enhanced by proteasome inhibitors as modeled in hemophilia A mice and dogs

Monahan[1] P.E., Sun[2] J., Lothrop Jr[3] C.D., Samulski[2] R.J.Pediatrics, Hematology Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

Although the delivery of genes that are larger than the size of the wild type adeno-associated virus genome (AAV, 4680 nt) is generally inefficient using AAV vectors, we have previously shown *in vitro* that concurrent proteasome inhibitor (PI) treatment improves transduction by oversize AAV vectors. AAV serotype 2 or 8 vectors carrying a 5.6 kb factor VIII cassette subsequently were studied in hemophilia A mice treated with or without PI. PI enhanced FVIII expression on average 6-fold using AAV2 and 3-fold with AAV8. Four hemophilia A dogs have now received a limiting dose of $1 \times 10^{13}$ vg/kg AAV8.canine FVIII via portal vein after receiving a single I.V. dose of PI; three hemophilic dogs received the same liver-directed vector without PI. No toxicity was observed, specifically no abnormalities of liver transaminases, blood platelet, white blood cell or other cell counts, or development of FVIII inhibitory antibodies. The Whole Blood Clotting Time (WBCT), normally prolonged to greater than 20 min in hemophilic dogs, corrected to the normal range (normal = 6–10 min) by the first timepoint at one week after vector infusion in the dogs receiving PI; dogs receiving vector without PI corrected only partially. Over the first 2 months after AAV+PI, 30 of 44 individual WBCT measurements in four dogs were normal, mean WBCT 9.7 min; three dogs receiving the same dose of AAV alone had only 1 of 32 normal assays, with a mean WBCT > 15 min during that time. Two of the dogs have now been followed for 27 months after AAV8.cFVIII + PI with 1–2 bleeds apiece and maintain a mean WBCT of 12 min; two littermates that received vector alone bled 12–14 times during the same period before each experiencing fatal hemorrhages. In summary, proteasome inhibitor enhanced sustained AAV-mediated expression of this large therapeutic transgene cassette in two preclinical animal models, enabling phenotypic correction of hemophilia with a beneficial safety profile.

Gene Ther. 2009 Jan;16(1):60-9. Epub 2008 Aug 14.
Enhancing transduction of the liver by adeno-associated viral vectors.
Nathwani AC, Cochrane M, McIntosh J, Ng CY, Zhou J, Gray JT, Davidoff AM.
Department of Haematology, UCL Cancer Institute, London, UK. a.nathwani@ucl.ac.uk
A number of distinct factors acting at different stages of the adeno-associated virus vector (AAV)-mediated gene transfer process were found to influence murine hepatocyte transduction. Foremost among these was the viral capsid protein. Self-complementary (sc) AAV pseudotyped with capsid from serotype 8 or rh.10 mediated fourfold greater hepatocyte transduction for a given vector dose when compared with vector packaged with AAV7 capsid. An almost linear relationship between vector dose and transgene expression was noted for all serotypes with vector doses as low as 1 x 10(7) vg per mouse (4 x 10(8) vg kg(-1)) mediating therapeutic levels of human FIX (hFIX) expression. Gender significantly influenced scAAV-mediated transgene expression, with twofold higher levels of expression observed in male compared with female mice. Pretreatment of mice with the proteasome inhibitor bortezomib increased scAAV-mediated hFIX expression from 4+/-0.6 to 9+/-2 microg ml(-1) in female mice, although the effect of this agent was less profound in males. Exposure of mice to adenovirus 10-20 weeks after gene transfer with AAV vectors augmented AAV transgene expression twofold by increasing the level of proviral mRNA. Hence, optimization of individual steps in the AAV gene transfer process can further enhance the potency of AAV-mediated transgene expression, thus increasing the probability of successful gene therapy.

Mol Ther. 2009 Nov 10. [Epub ahead of print]
Proteasome Inhibitors Decrease AAV2 Capsid derived Peptide Epitope Presentation on MHC Class I Following Transduction.
Finn JD, Hui D, Downey HD, Dunn D, Pien GC, Mingozzi F, Zhou S, High KA.
Department of Pediatrics, Division of Hematology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA.
Adeno-associated viral (AAV) vectors are an extensively studied and highly used vector platform for gene therapy applications. We hypothesize that in the first clinical trial using AAV to treat hemophilia B, AAV capsid proteins were presented on the surface of transduced hepatocytes, resulting in clearance by antigen-specific CD8(+) T cells and consequent loss of therapeutic transgene expression. It has been previously shown that proteasome inhibitors can have a dramatic effect on AAV transduction in vitro and in vivo. Here, we describe using the US Food and Drug Administration-approved proteasome inhibitor, bortezomib, to decrease capsid antigen presentation on hepatocytes in vitro, whereas at the same time, enhancing gene expression in vivo. Using an AAV capsid-specific T-cell reporter (TCR) line to analyze the effect of proteasome inhibitors on antigen presentation, we demonstrate capsid antigen presentation at low multiplicities of infection (MOIs), and inhibition of antigen presentation at pharmacologic levels of bortezomib. We also demonstrate that bortezomib can enhance Factor IX (FIX) expression from an AAV2 vector in mice, although the same effect was not observed for AAV8 vectors. A pharmacological agent that can enhance AAV transduction, decrease T-cell activation/proliferation, and decrease capsid antigen presentation would be a promising solution to obstacles to successful AAV-mediated, liver-directed gene transfer in humans.
PMID: 19904235 [PubMed - as supplied by publisher]

1: Blood. 2004 Feb 15;103(4):1253-60. Epub 2003 Oct 9.

**Total correction of hemophilia A mice with canine FVIII using an AAV 8 serotype.**

**Sarkar R, Tetreault R, Gao G, Wang L, Bell P, Chandler R, Wilson JM, Kazazian HH Jr.**

Department of Genetics, University of Pennsylvania 415 Curie Blvd, CRB Rm 475, Philadelphia, PA 19104, USA. sarkarr@mail.med.upenn.edu

Despite the popularity of adeno-associated virus 2 (AAV2) as a vehicle for gene transfer, its efficacy for liver-directed gene therapy in hemophilia A or B has been suboptimal. Here we evaluated AAV serotypes 2, 5, 7, and 8 in gene therapy of factor VIII (FVIII) deficiency in a hemophilia A mouse model and found that AAV8 was superior to the other 3 serotypes. We expressed canine B domain-deleted FVIII cDNA either in a single vector or in 2 separate AAV vectors containing the heavy- and light-chain cDNAs. We also evaluated AAV8 against AAV2 in intraportal and tail vein injections. AAV8 gave 100% correction of plasma FVIII activity irrespective of the vector type or route of administration.

PMID: 14551134 [PubMed - indexed for MEDLINE]

1: Blood. 2004 Jan 1;103(1):85-92. Epub 2003 Sep 11.

**Safety and efficacy of factor IX gene transfer to skeletal muscle in murine and canine hemophilia B models by adeno-associated viral vector serotype 1.**

**Arruda VR, Schuettrumpf J, Herzog RW, Nichols TC, Robinson N, Lotfi Y, Mingozzi F, Xiao W, Couto LB, High KA.**

Department of Pediatrics, University of Pennsylvani Medical Center, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

Adeno-associated viral (AAV) vectors (serotype 2) efficiently transduce skeletal muscle, and have been used as gene delivery vehicles for hemophilia B and for muscular dystrophies in experimental animals and humans. Recent reports suggest that AAV vectors based on serotypes 1, 5, and 7 transduce murine skeletal muscle much more efficiently than AAV-2, with reported increases in expression ranging from 2-fold to 1000-fold. We sought to determine whether this increased efficacy could be observed in species other than mice. In immunodeficient mice we saw 10- to 20-fold higher levels of human factor IX (hF.IX) expression at a range of doses, and in hemophilic dogs we observed approximately 50-fold higher levels of expression. The increase in transgene expression was due partly to higher gene copy number and a larger number of cells transduced at each injection site. In all immunocompetent animals injected with AAV-1, inhibitory antibodies to F.IX developed, but in immunocompetent mice treated with high doses of vector, inhibitory antibodies eventually disappeared. These studies emphasize that the increased efficacy of AAV-1 vectors carries a risk of inhibitor formation, and that further studies will be required to define doses and treatment regimens that result in tolerance rather than immunity to F.IX.

PMID: 12969984 [PubMed - indexed for MEDLINE]

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Theodore E. Woodward Award. AAV-mediated gene transfer for hemophilia

AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B.

Efficient induction of immune tolerance to coagulation factor IX following direct intramuscular gene transfer

Therapeutic levels of factor IX expression using a muscle-specific promoter and adeno-associated virus

AAV-mediated gene transfer for hemophilia.

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Long-term correction of inhibitor-prone hemophilia B dogs treated with liver-directed AAV2-mediated factor IX

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Total correction of hemophilia A mice with canine FVIII using an AAV 8 serotype.

Sarkar and 2004 and Blood... (1)

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1: Hum Gene Ther. 2006 Aug;17(8):807-20.

**Enhanced gene transfer efficiency in the murine striatum and an orthotopic glioblastoma tumor model, using AAV-7- and AAV-8-pseudotyped vectors.**

**Harding TC, Dickinson PJ, Roberts BN, Yendluri S, Gonzalez-Edick M, Lecouteur RA, Jooss KU.**

Cell Genesys, South San Francisco, CA 94080, USA. thomash@cellgenesys.com

In this study, recombinant AAV vectors pseudotyped with viral capsids derived from AAV serotypes 7 and 8 were evaluated for gene transfer in the murine striatum relative to vectors pseudotyped with AAV serotypes 2, 5, and 6. In comparison with rAAV serotype 2, pseudotyped vectors derived from AAV-7 and AAV-8 have increased transduction efficiency in the murine CNS, with the rank order rAAV-7 > rAAV-8 > rAAV-5 > rAAV-2 = rAAV-6, with all vectors demonstrating a marked tropism for neuronal transduction. Pseudotyped rAAV vector gene transfer in the brain after preimplantation of a murine 4C8 glioblastoma tumor was also evaluated. Efficiency of gene transfer to the orthotopic tumor was increased when using AAV-6, -7, and -8 capsid proteins in comparison with serotype 2, with the order rAAV-8 = rAAV-7 > rAAV-6 > rAAV-2 > rAAV-5. The increased gene transfer efficiency of rAAV vectors pseudotyped with the rAAV-8 capsid also provided enhanced therapeutic efficacy in a mouse model of glioblastoma multiforme, using vectors encoding an inhibitor of the vascular endothelial growth factor pathway. These studies demonstrate that rAAV vectors pseudotyped with capsids derived from AAV serotypes 7 and 8 provide enhanced gene transfer in the murine CNS and may offer increased therapeutic efficacy in the treatment of neurological disease.

PMID: 16942441 [PubMed - indexed for MEDLINE]

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AAV serotype 8-mediated gene delivery of a soluble VEGF receptor to the CNS for the treatment of

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1: Gene Ther. 2005 Oct;12(20):1534-8.

**Adeno-associated virus (AAV)-7 and -8 poorly transduce vascular endothelial cells and are sensitive to proteasomal degradation.**

**Denby L, Nicklin SA, Baker AH.**

British Heart Foundation, Glasgow Cardiovascular Research Centre, Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.

Transduction of the vascular endothelium by adeno-associated virus (AAV) vectors would have broad appeal for gene therapy. However, levels of transduction by AAV serotype-2 are low, an observation linked to deficiencies in endothelial cell binding, sequestration of virions in the extracellular matrix and/or virion degradation by the proteasome. Strategies to improve transduction of endothelial cells include AAV-2 capsid targeting using small peptides isolated by phage display or the use of alternate serotypes. Previously, we have shown that AAV serotypes-3 through -6 transduce endothelial cells with poor efficiency. Recently, AAV serotypes-7 and -8 have been shown to mediate efficient transduction of the skeletal muscle and liver, respectively, although their infectivity profile for vascular cells has not been addressed. Here, we show that AAV-7 and -8 also transduce endothelial cells with poor efficiency and the levels of transgene expression are markedly enhanced by inhibition of the proteasome. In both cases proteasome blockade enhances the nuclear translocation of virions. We further show that this is vascular cell-type selective since transduction of smooth muscle cells is not sensitive to proteasome inhibition. Analysis in intact blood vessels corroborated these findings and suggests that proteasome degradation is a common limiting factor for endothelial cell transduction by AAV vectors.

PMID: 15944729 [PubMed - indexed for MEDLINE]

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1: J Virol. 2001 Feb;75(4):1824-33.



**Intracellular trafficking of adeno-associated virus vectors: routing to the late endosomal compartment and proteasome degradation.**

**Douar AM, Poulard K, Stockholm D, Danos O.**

Genethon III-CNRS URA 1923, Evry, France. douar@genethon.fr

The early steps of adeno-associated virus (AAV) infection involve attachment to a variety of cell surface receptors (heparan sulfate, integrins, and fibroblast growth factor receptor 1) followed by clathrin-dependent or independent internalization. Here we have studied the subsequent intracellular trafficking of AAV particles from the endosomal compartment to the nucleus. Human cell lines were transduced with a recombinant AAV (rAAV) carrying a reporter gene (luciferase or green fluorescent protein) in the presence of agents that affect trafficking. The effects of bafilomycin A(1), brefeldin A, and MG-132 were measured. These drugs act at the level of endosome acidification, early-to-late endosome transition, and proteasome activity, respectively. We observed that the transducing virions needed to be routed as far as the late endosomal compartment. This behavior was markedly different from that observed with adenovirus particles. Antiproteasome treatments with MG-132 led to a 50-fold enhancement in transduction efficiency. This effect was accompanied by a 10-fold intracellular accumulation of single-stranded DNA AAV genomes, suggesting that the mechanism of transduction enhancement was different from the one mediated by a helper adenovirus, which facilitates the conversion of the rAAV single-stranded DNA genome into its replicative form. MG-132, a drug currently in clinical use, could be of practical use for potentializing rAAV-mediated delivery of therapeutic genes.

PMID: 11160681 [PubMed - indexed for MEDLINE]